TM 702 – COOKED MEAT MEDIUM (R.C. MEDIUM) (MEAT GRANULES)

INTENDED USE

For cultivation of aerobes and anaerobes especially pathogenic Clostridia and also for maintenance of stock cultures.

PRODUCT SUMMARY AND EXPLANATION

Clostridium is a large genus of gram-positive spore-bearing anaerobes. They are normally present in soil, some are responsible for human and animal diseases and others are associated with food spoilage. They may be saccharolytic, decomposing sugars to form butyric and acetic acids and alcohols. The beef heart, solids in Robertson's Medium is reddened and gas is produced. Other proteolytic species attack the amino acids. Beef Heart, solids in Robertson's medium is blackened and decomposed by *Clostridium* species, giving the culture a foul odour. The mesophilic spore-forming anaerobes are of primary importance in the spoilage of low acid foods packed in sealed containers, because of their high heat resistance, their ability to grow in the absence of oxygen and a growth range which covers the temperature of normal storage of canned and other processed foods including the refrigerated storage of cured meats.

Cooked M-Medium was originally developed by Robertson for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped M-Medium, which supports the growth of many spore forming and non-spore forming strict anaerobes. It has the ability to initiate growth of bacteria from very small inocula and to maintain the viability of cultures over long period. Mixed cultures of bacteria survive in Cooked M-Medium without displacing the slower-growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic organisms. It is used for cultivation and maintenance of Clostridia and for determining proteolytic activity of anaerobes. FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods.

COMPOSITION

Ingredients	Gms / Ltr
Sodium chloride	5.000
Protease peptone	5.000
Dextrose (Glucose)	2.000
Beef Heart, solids	98.000

PRINCIPLE

Cooked M-Medium contains Beef heart, solids, which provide amino acids and other nutrients. It also contains glutathione, a reducing substance that permits the growth of obligate anaerobes. The sulfhydryl groups, which impart reducing effect, are more available in denatured protein and hence cooked meat is added in the medium. The addition of dextrose allows rapid and heavy growth of anaerobic bacteria in a short time and leads to more rapid identification of important anaerobes. Growth in this medium is indicated by turbidity or bubble formation by some organisms. Blackening and disintegration of the meat particles indicate proteolysis. For best results, medium should be used on the day it is prepared, otherwise it should be boiled or steamed for a few minutes and allowed to cool without agitation and then inoculated. Inoculation should be made near the bottom of the tube in the meat particles for anaerobic cultures. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium. For the isolation of *Clostridium* from food, use a stomacher to prepare 10% suspension of the food in Peptone Water diluent. Make dilutions and plate, both suspensions and dilutions on Willis and Hobbs Medium Base, Tryptose Sulphite Cycloserine (T.C.S.) Agar Base. Place a metronidazole disc on the inoculum. Incubate anaerobically at 37°C overnight. To count the clostridia, pour the plates with the dilutions on Perfringens Agar Base (O.P.S.P.). Incubate duplicate plates aerobically and anaerobically to

distinguish between clostridia and other organisms. Add some of the suspension to two tubes of Cooked Medium. Heat one tube for 10 min at 80°C and incubate as above. Growth of clostridia is visualized as turbidity or gas bubbles. This medium can be further tested for presence of *Clostridium.*

INSTRUCTION FOR USE

- Dissolve 12.5 grams in 100 ml purified/distilled water (or suspend 1.25 grams in 10 ml distilled water in test tubes).
- Mix thoroughly and allow to stand for 15 minutes until all the particles are thoroughly wetted.
- Dispense into tubes or flasks as desired.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Brown coloured granules.
Appearance of prepared medium : Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.
pH (at 25°C) : 7.2 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Clostridium botulinum	25763	50-100	Luxuriant	35-37°C	40-48 Hours
Clostridium perfringens	12924	50-100	Luxuriant	35-37°C	40-48 Hours
Enterococcus faecalis	29212	50-100	Luxuriant	35-37°C	40-48 Hours
Streptococcus pneumoniae	6303	50-100	Luxuriant	35-37°C	40-48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

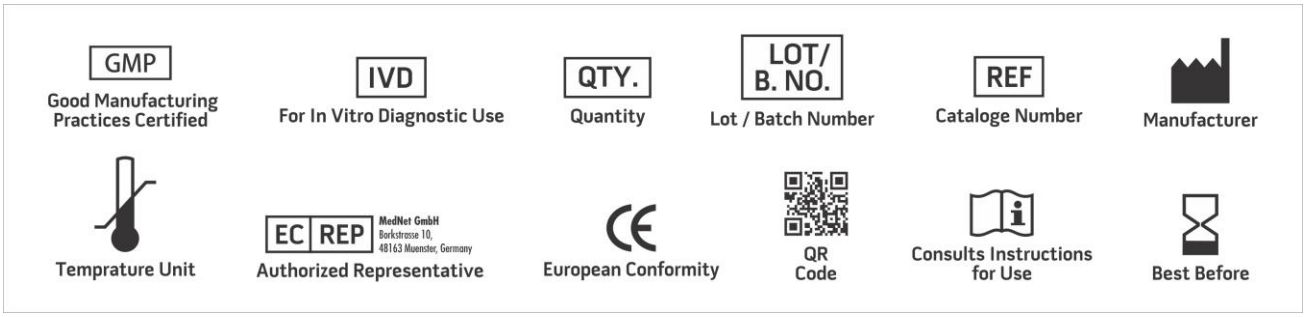
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. MacFaddin J. F., 1985, Media for Isolation - Cultivation - Identification - Maintenance of Medical bacteria, Vol. I, Williams& Wilkins, Baltimore.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology,8th Ed., American Society for Microbiology, Washington, D.C.
3. U. S. Food and Drug Administration, 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va.
4. CollinsC.H.,LyneP.M.,GrangeJ.M.,1985,7thEd.,MicrobiologicalMethods.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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